4. HIGH TEMPERATURE EFFECT ON THE POLLEN GRAINS OF THREE VARIETIES OF CULTIVATED MAIZE

M. KEDVES, A. TÓTH, K. MÉSZÁROS, A. BORBOLA, O. BENN and T. GUBÁS

Cell Biological and Evolutionary Micropaleontological Laboratory of the Department of Botany of the J. A. University, H-6701, P. O. Box 993, Szeged, Hungary

Abstract

The high temperature effect on the pollen grains of the open pollinated local varieties of cultivated maize was investigated with the LM method: Chapalote, Canario de Ocho and St. Croix Long Ear. The experiments were made on 200 °C temperature from 10' until 300 hrs. The qualitative alterations are essentially identical at the different varieties. The originally isodiametric, monoporate pollen grains altered secondarily to monosulcate, trichotomo- tetratomosulcate forms, several secondary ones assimilate to the pollen grains of the *Cyperaceae.* Among the alterations of the quantitative characters, differences were established between the investigated varieties. The presented results are important from the point of view of the evolution of the *angiosperm* pollen grains and of the taxonomical evaluation of the fossil pollen grains of *"Gramineae*-type".

Key words: Palynology, recent, high temperature effect, maize varieties.

Introduction

This contribution present part of the research program of our laboratory, namely the secondary alterations of the recent spores and pollen grains. Based on our previous published results, the alterations of the pollen grains in consequence of the high temperature may be important from the point of views as follows.

1. The quantitative alterations may be useful in the investigation of the organic material of the metamorphic sediments. In some cases the diameter and the size of the morphological characteristic features may be important in the determination of the fossil sporomorphs. Some characteristic features may altered in consequence of the sedimentation processes.

2. The qualitative alterations are useful from taxonomical and evolutionary point of view. Moreover, the experimentally produced secondary forms may be taken into consideration also at the evaluation of the fossil forms. At our first experiments (KEDVES and KINCSEK, 1989) early morphological characteristic features appeared at the pollen grains of *Corylus avellana* L. and *Betula verrucosa* L. after the high temperature effect. Later the opposite alteration has also been established. The secondary forms of the recent inaperturate pollen grains of *Taxus baccata* L. and *Juniperus virginiana* L. may be similar among others to the tricolpate earliest *an-*

giosperm pollen grains (KEDVES, TÓTH and FARKAS, 1991). It is possible, that among the described first Lower Cretaceous *angiosperm* pollen grains there are secondary forms of inaperturate *gymnosperm* pollen grains. TEM investigations are needed to solve this problem.

3. The evolutionary degrees of the *angiosperm* pollen grains in the Northern Hemisphere are based on the schemes of DOYLE (1977). The most important evolutionary stages are as follows. Monosulcate – tricolpate – tricolporate – *Normapolles*. This scheme is restricted to Europe, and the Atlantic Coast of North America. Based on new data, another, parallel lineage may also be presumed, as follows: The exine ultrastructure of the mesozoic isodiametric *gymnosperm Spheripollenites* COUPER 1958 is essentially of the *angiosperm* type (KEDVES and PÁRDUTZ, 1973). The LM studies demonstrated a non-characteristic pore-like aperture. This kind of *angiosperm* pollen evolutionary lineage may be originated from the *gymnosperms Spheripollenites* type and termined at the monoporate *angiosperm* pollen type, e. g.: *Gramineae, Restionaceae.*

Basically the aim was to investigate the secondary alterations of the monoporate pollen type. Moreover for the first time a cultivated species with three varieties was chosen for this kind of investigation. In this way the second point of view was to investigate the pollen morphology and the alterations of three local varieties within one cultivated species.

Materials and Methods

The pollen material was collected by Dr. A. PALÁGYI on 23. 08. 1991. Locality: Ságvári Experimental Research Station of the Cereal Research Institute. The pollen grains were frozen at –20 °C after collection. The experiments were made or started on 04. 09. 1991. Temperature 200 °C, length of time and numbers of experiments are as follows.

Lenght of time	Experiment No		
	Chapalote	Canario de Ocho	St. Croix Long Ear
0'	1217	1218	1219
10'	1220	1221	1222
1 h.	1223	1224	1225
5 hrs.	1226	1227	1228
10 hrs.	1229	1230	1231
25 hrs.	1232	1233	1234
50 hrs.	1235	1236	1237
100 hrs.	1238	1239	1240
200 hrs.	1241	1242	1243
300 hrs.	1244	1245	1246

The slides for light-microscopical investigations were mounted in glycerine-jelly hydrated at 39.6%. 200 specimens of each sample were investigated qualitatively and quantitatively. The pictures were taken with an objective Carl Zeiss Jena, GF Planachromat HI 100X/1.25/0.17–A.

General problems

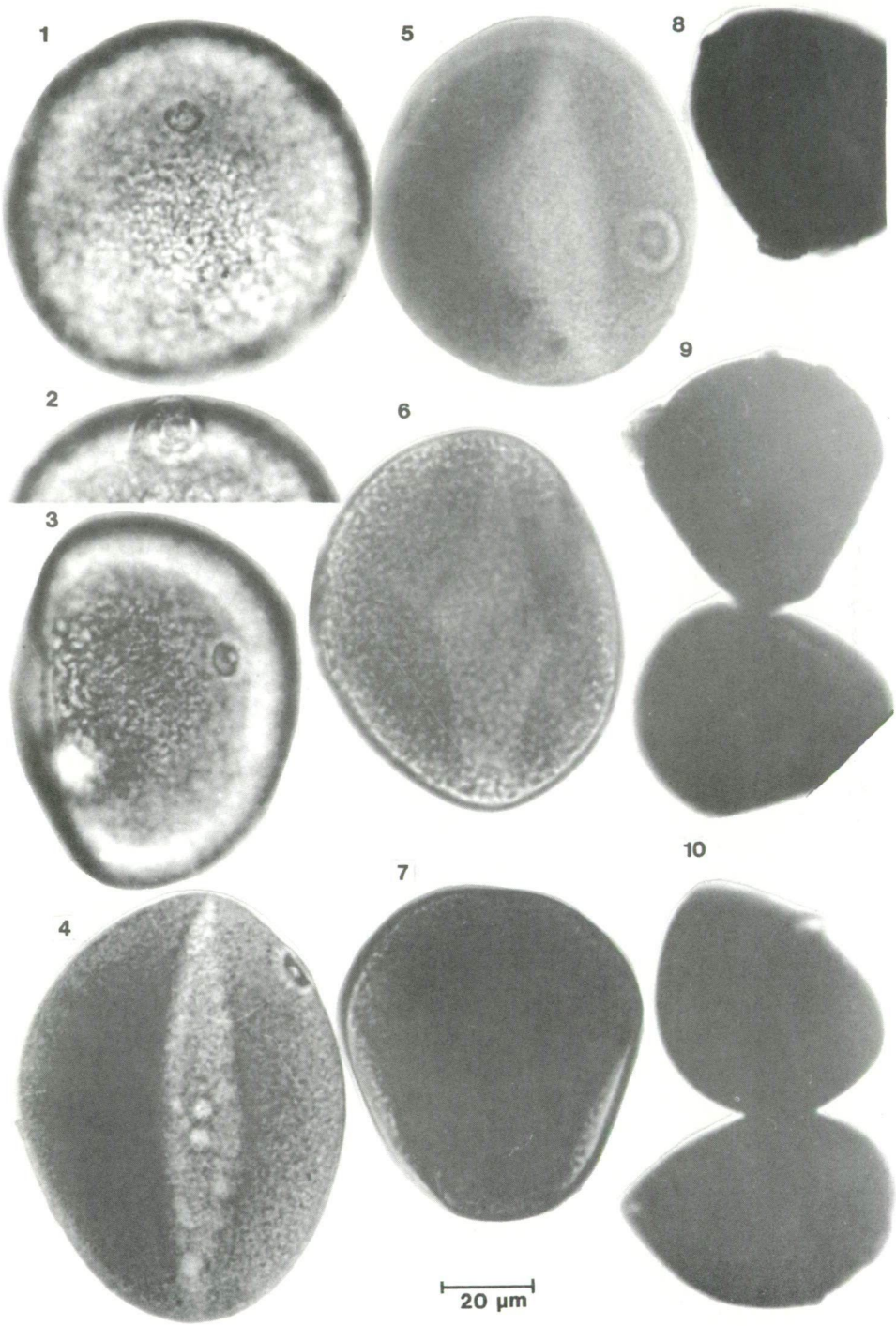
There is a number of palynological data about the pollen grains of the genus *Zea* L. Most of them are enumerated in the volumes of "Index bibliographique sur la morphologie des pollens d'Angiospermes", cf. THANIKAIMONI (1972, 1973, 1976, 1980, 1986). TISSOT (1990) and TISSOT and VAN DER HAM (1994). Based on the above mentioned compilations, as the earliest data the papers of MOHL (1834, 1835), HASSAN (1842) and FISHER (1890) can be pointed out.

The pollen grains of maize are typically of *"Gramineae* type"; spherical, monoporate, pore with operculum, and surrounded by annulus. Because of the agricultural importance, and the intensive experimental work to produce new local varieties or hybrids, it is important to publish full taxonomical data in every paper containing pollen data. Palynological data with the determination of *Zea mays* L. are only insufficient.

HUYNH (1975) established that the reproduction organells of the *Gramineae* are peculiar in several points of view: 1. The sporogene tissue is reduced to one single layer of mother cells. The arrangement of the microspores is isobilateral, etc. On microspore level at the cultivated maize SZAKÁCS (1992) described pollen dimorphism. "Dark" and "clear" microspores were distinguished: their size is identical, but there are differences in the structure of the cytoplasm. Within the problem of the pollen – stigma interaction of the grasses, the tissue organisation and the cytochemistry of the stigma of *Zea mays* L. was investigated by HESLOP-HARRISON, Y., REGER, and HESLOP-HARRISON, J. (1984). GRANT (1972) investigated with the SEM method the *Zea mays* L. ssp. *mays, Z. mays* L. ssp. *mexicana* (SCHRAD.) ILTIS (teosinte), *Zea perennis* (HITCHC.) REEVES and MANGELDORF (perennial teosinte), and two species of *Tripsacum* L. Based on the SEM data the *Zea* taxa pollen grains are similar pollen types, spinules scattered regularly over the exine surface. The surface of the *Tripsacum* species is characterized by distinctly reticuloid pattern with spinules on isolated lacunae. Hybrids between *Zea* and *Tripsacum* are intermediate in exine pattern or similar to *Tripsacum* depending on the genome combination. TSUKADA and ROWLEY (1964) summarized the three methods to distinguish maize pollen from that of other grasses: 1. The size (FIRBAS, 1937), 2. The ratio of the dimensions of the annulus and pore related to the size of the pollen grain (BARGHOORN, WOLFE and CLISBY, 1954), 3. The pattern of the spinules and infratectal

Plate 4.1. ►

1–10. *Zea mays* L. l. var. Chapalote, Recent.
1,2. Pollen grains without heating.
3. Experiment No: 1220, length of time 10 min.
4,5. Experiment No: 1223, length of time 1 hr.
6. Experiment No: 1226, length of time 5 hrs.
7. Experiment No: 1229, length of time 10 hrs.
8. Experiment No: 1238, length of time 100 hrs.
9. Experiment No: 1241, length of time 200 hrs.
10. Experiment No: 1244, length of time 300 hrs.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elements (columellae) (ERDTMAN, 1956, etc.). Finally they established fossil maize pollen from Guatemala. BARTLETT, BARGHOORN and BERGER (1969) based on their palynological studies demonstrated wild maize, agricultural maize, and Manihot from about 7.300 years old sediments in the Gatun Basin, Panama.

The above mentioned few selected bibliographical data verified the complexity of the problem of the maize pollen grains and its importance.

Results

QUALITATIVE DATA

The LM microscopy revealed differences between fresh pollen grains of the investigated three local varieties. Based on our present data, two pollen morphotypes can be established:

1. Chapalote and St. Croix Long Ear
2. Canario de Ocho

SEM and TEM data are necessary to establish the fine differences between the above mentioned two types, because using the LM method we observed differences in the organelles of the protoplasm, and in the surface ornamentation also.

The high temperature effect resulted in the following secondary forms:

Monocolpate (monosulcate), (Plate 4.1., figs. 3,4, plate 4.3., figs. 5,6).

Trichotomosulcate (Plate 4.1., fig. 5, plate 4.2., fig. 7, plate 4.3., fig. 7).

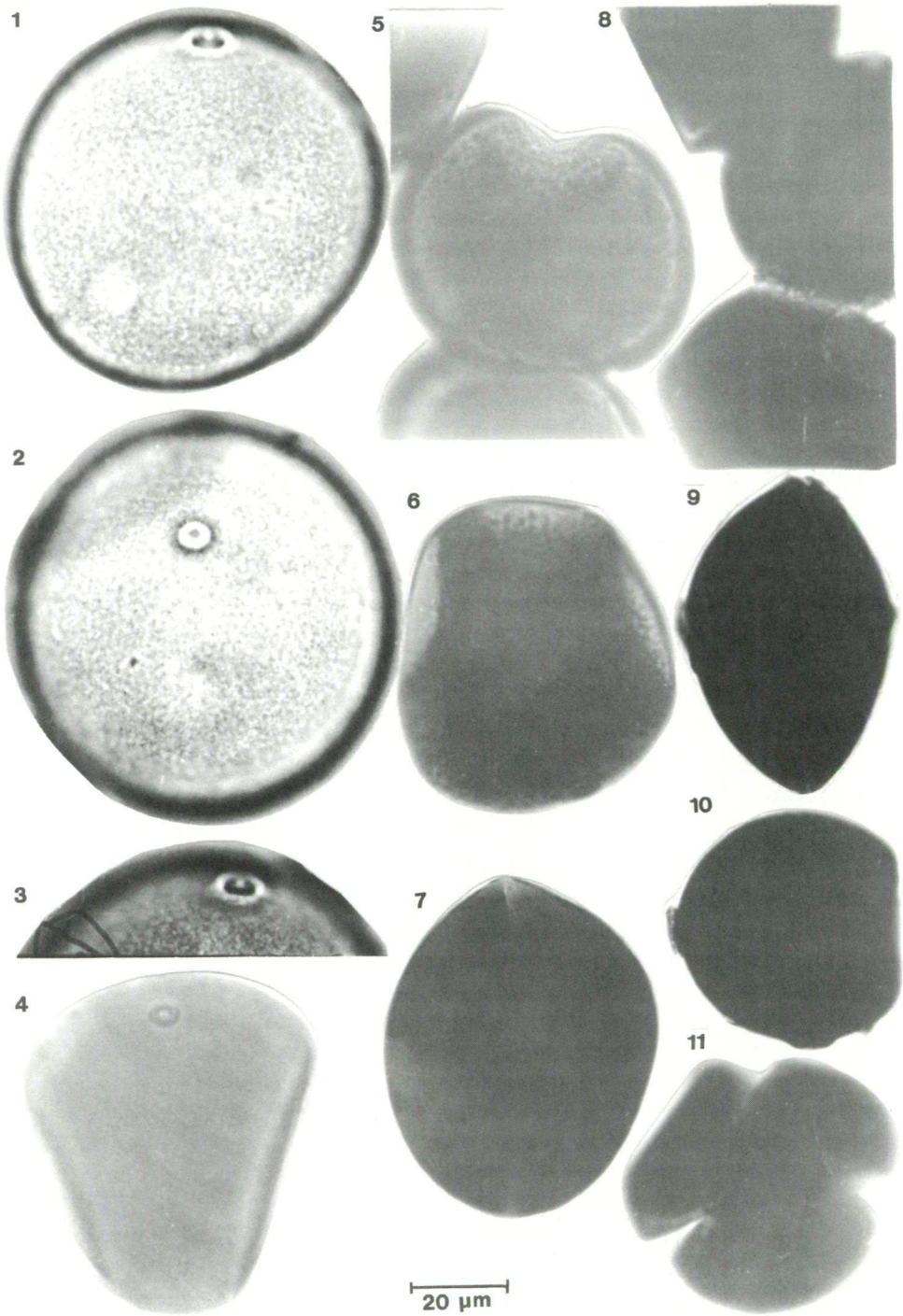
Tetratomosulcate (Plate 4.1., fig. 6, plate 4.3., figs. 4,6).

"Cyperaceae-like forms" (Plate 4.1., fig. 7, plate 4.2., fig. 6).

Opaque, very damaged forms (Plate 4.1., figs. 8–10, plate 4.2., figs. 8–11, plate 4.3., figs. 8–10).

Plate 4.2.

1–11. *Zea mays* L. l. var. Canario de Ocho, Recent.
1–3. Pollen grains without heating.
4. Experiment No: 1224, length of time 1 hr.
5. Experiment No: 1227, length of time 5 hrs.
6. Experiment No: 1230, length of time 10 hrs.
7. Experiment No: 1233, length of time 25 hrs.
8. Experiment No: 1236, length of time 50 hrs.
9. Experiment No: 1239, length of time 100 hrs.
10. Experiment No: 1242, length of time 200 hrs.
11. Experiment No: 1245, length of time 300 hrs.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The per cents of the different secondary forms by the local varieties

Zea mays L. 1. var. Chapalote

Per cents of the pollen forms	Length of time									
	0	10'	1^h	5^h	10^h	25^h	50^h	100^h	200^h	300^h
Non-altered	100.0	84.5	37.5	36.5	22.5	12.5	18.5	19.5	32.5	32.0
Monocolpate		9.5	43.5	42.0	45.0	48.0	46.0	46.0	37.5	42.5
Cyperaceae-like		6.0	17.5	15.0	19.5	24.0	21.5	22.0	21.0	17.5
Trichotomosulcate			1.5	6.5	12.5	14.0	14.0	12.5	9.0	8.0
Tetratomosulcate					0.5	1.5				

Zea mays L. 1. var. Canario de Ocho

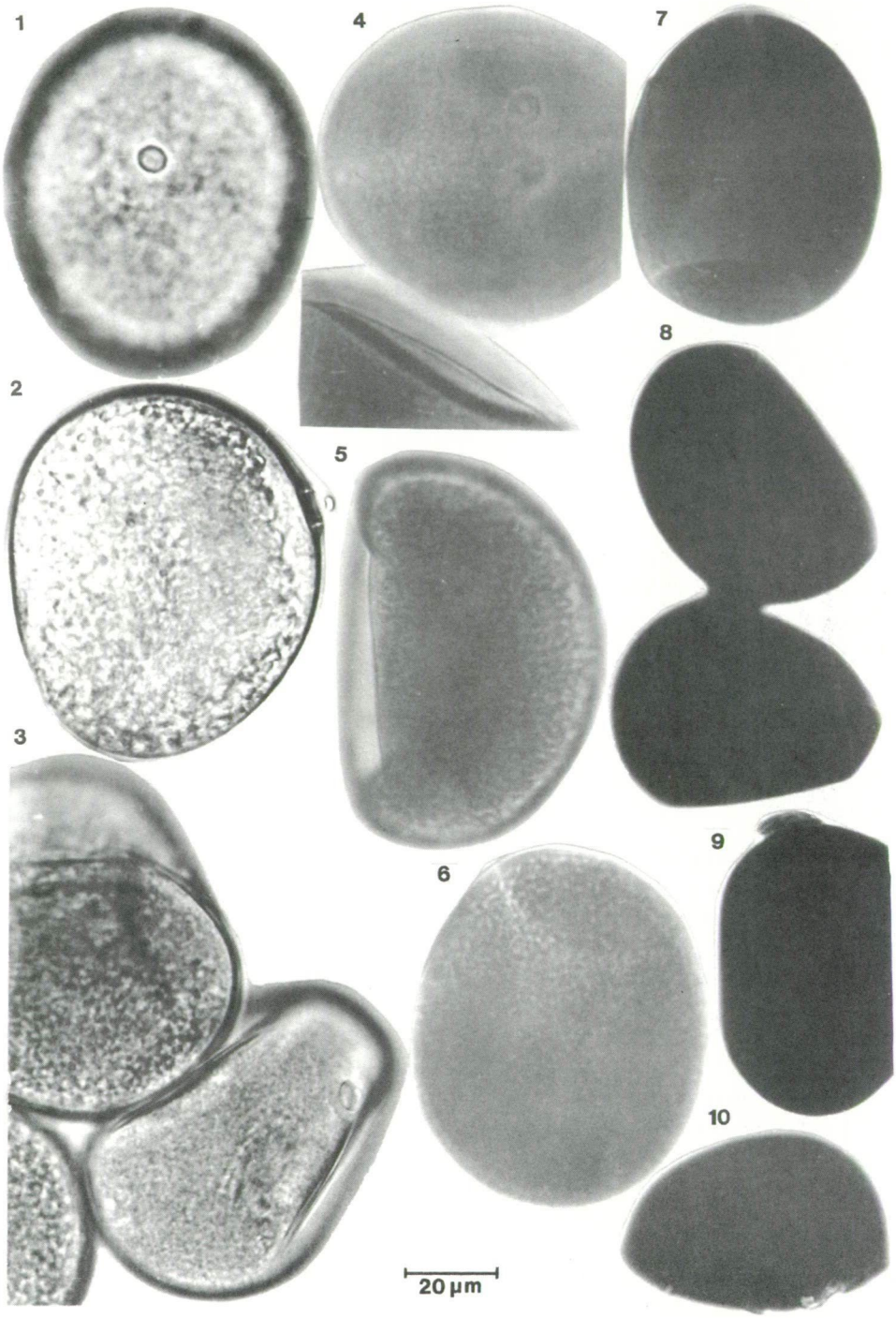
Per cents of the pollen forms	Length of time									
	0	10'	1^h	5^h	10^h	25^h	50^h	100^h	200^h	300^h
Non-altered	100.0	91.0	40.0	38.0	17.5	17.5	21.0	20.5	26.0	23.5
Monocolpate		6.5	36.5	41.0	47.0	31.5	35.0	39.5	37.0	42.5
Cyperaceae-like		2.5	22.0	13.5	23.0	25.5	28.5	25.5	31.0	21.0
Trichotomosulcate			1.5	7.5	11.5	23.0	15.5	14.5	6.0	12.5
Tetratomosulcate					1.0	2.5				0.5

Zea mays L. 1. var. St. Croix Long Ear

Per cents of the pollen forms	Length of time									
	0	10'	1^h	5^h	10^h	25^h	50^h	100^h	200^h	300^h
Non-altered	100.0	86.5	27.5	30.5	15.5	7.0	13.0	17.5	24.0	27.5
Monocolpate		10.0	44.5	50.0	50.5	51.0	48.5	49.0	50.0	49.5
Cyperaceae-like		3.5	15.5	15.0	18.0	26.0	23.0	24.5	20.0	13.5
Trichotomosulcate			7.5	4.0	9.0	10.5	15.5	9.0	6.0	9.0
Tetratomosulcate			5.0	0.5	7.0	5.5				0.5

Plate 4.3. ►

1–10. *Zea mays* L. l. var. St. Croix Long Ear, Recent.
1,2. Pollen grains without heating.
3. Experiment No: 1222, length of time 10 min.
4. Experiment No: 1225, length of time 1 hr.
5. Experiment No: 1228, length of time 5 hrs.
6. Experiment No: 1231, length of time 10 hrs.
7. Experiment No: 1234, length of time 25 hrs.
8,9. Experiment No: 1237, length of time 50 hrs.
10. Experiment No: 1240, length of time 100 hrs.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Regarding the distribution of the percentages of the different pollen forms, the following can be pointed out:

1. At the investigated three local varieties of *Zea mays* L. the fresh pollen grains are more or less isodiametric, monoporate type.
2. Monosulcate (colpate) and *Cyperaceae*-like secondary forms appeared after 10' of heating at 200 °C in a remarkable per cent.
3. The pollen grains heated for one hour altered secondarily in a remarkable proportion. The amount of the monocolpate + *Cyperaceae*-like forms is more than 50% (43.5 + 17.5, 36.5 + 22.0, 44.5 + 15.5). Secondarily trichotomosulcate forms are relatively few, 1.5 at the varieties Chapalote and Canario de Ocho, higher, 7.5% at the variety St. Croix Long Ear. At this latter mentioned local variety tetratomosulcate secondary forms also appeared. This secondary form was observed in a remarkable quantity at this variety only after heating for 1–25 hours.
4. From 5 hours until 300 hours of heating the monocolpate forms are dominant. The so-called "normal monoporate forms" are similar or nearly in equal proportion to the *Cyperaceae*-like forms.
5. To the presented percentages it is necessary to emphasize that after 25 hours of heating the colour of the pollen grains is extremely dark, in this way sometimes it was not easy to establish the character of the secondary forms.
6. After 200 hours of heating several further secondary alterations appeared. The apertural area became very proeminent, this is more characteristic after 300 hours, accompanied by broken forms (Plate 4.2., fig. 11). Another alteration is when pollen grains are in pairs (Plate 4.1., figs. 10,11, plate 4.2., fig. 8, plate 4.3., fig. 8).

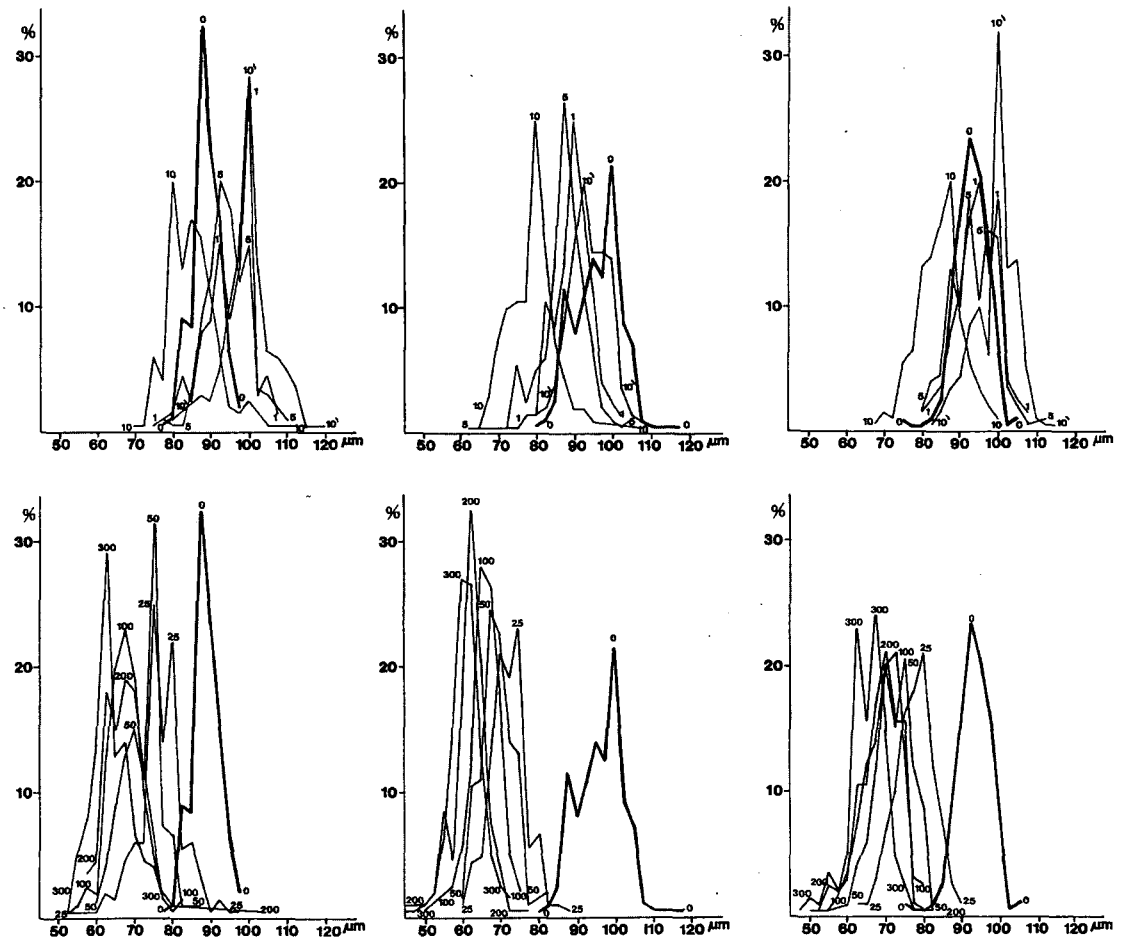
QUANTITATIVE DATA
(Text-figs. 4.1. and 4.2.)

1. Regarding the largest size of the investigated varieties, there are differences. The 2nd variety (Canario de Ocho) is the largest, the smaller is the 3rd (St. Croix Long Ear). At this latter mentioned variety the shape is also different in a remarkable quantity there are ellipsoidal forms also.
2. After 10' of heating the pollen grains of the 1st and the 3rd varieties started to swell, in contrast to this the originally largest pollen grains of the 2nd variety start to contract. Similar alterations happened in the P/A ratio in this case this is the ratio of the longest and the shortest dimension of the pollen grains. The data are as follows.

2.1. 1. var. Chapalote 1.0–1.9
2.2. 1. var. Canario de Ocho 1.0–1.4
2.3. 1. var. St. Croix Long Ear 1.0–1.9

3. Heating for 1 hour resulted at all varieties contraction. But the size at the 1st and the 3rd varieties does not reach the size of the pollen grains because in the first phase of heating these pollen grains have been swelled.
4. Results after heating for 5 hours. The size of the first variety has not reached the original size of the fresh pollen grain. It is interesting that the size of the 3rd variety is identical after this heating to the fresh pollen grain.

5. Heating for 10 hours resulted in a characteristic contraction at every varieties investigated. This continues after heating for 25 hours.
6. From 50 hours of heating the colour of the pollen grains is very dark, in some cases the determination of the morphological characteristic features is not easy.

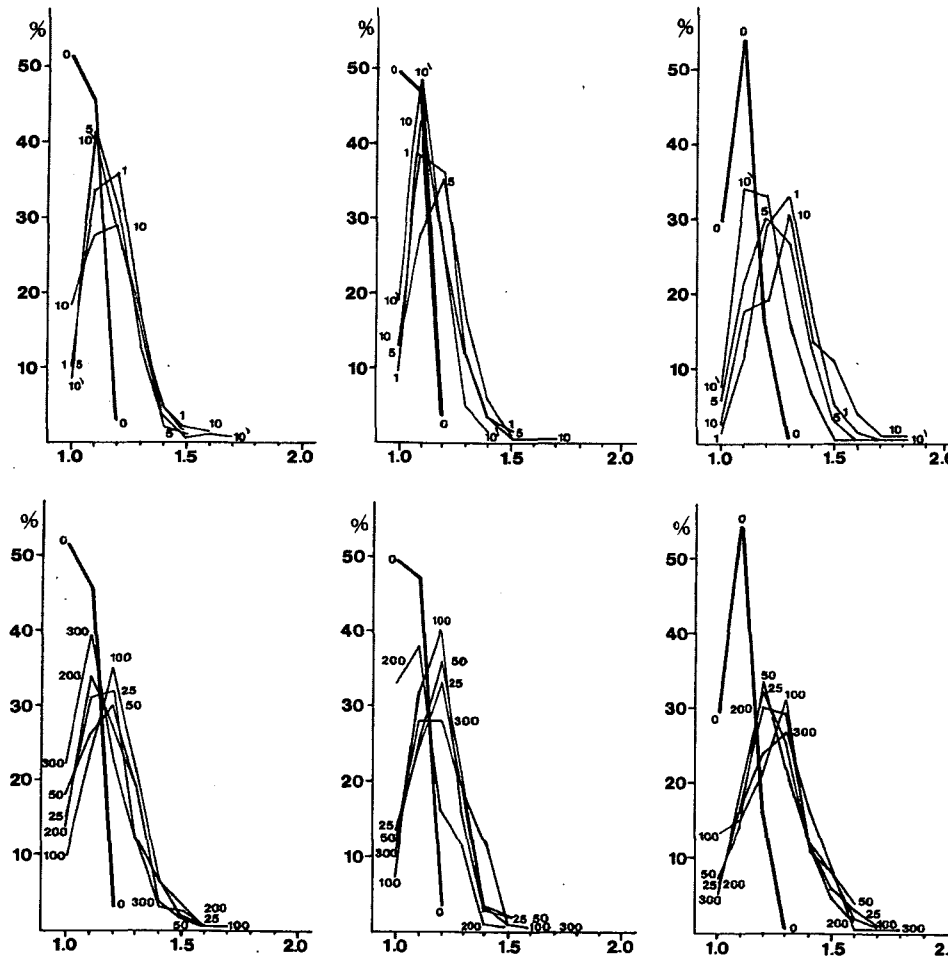


Text-fig. 4.1.

Variation-statistical diagrams of the diameter and/or the longest size of the pollen grains. From left to right: *Zea mays* L. l. var. Chapalote, *Zea mays* L. l. var. Canario de Ocho, *Zea mays* L. var. St. Croix Long Ear.

7. After heating for 100–200 and 300 hours, the decrease of the size of the pollen grains continues, but after 200 hours the diminution of the diameter is not so definite.

Finally, the alteration of the P/A ration is one thing to emphasize that the 1st and the 2nd varieties are similar, the 3rd is quite different from the previous ones.



Text-fig. 4.2.

Variation-statistical diagrams of the secondary P/E ratio of the pollen grains. From left to right: *Zea mays* L. l. var. Chapalote, *Zea mays* L. l. var. Canario de Ocho, *Zea mays* L. var. St. Croix Long Ear.

Discussion and Conclusions

On the basis of our recent results we can conclude the following:

1. There are remarkable differences in some of the morphological characteristic features of the pollen grains of the local varieties investigated. These differences appeared at the experimentally altered forms too. As it was emphasized previously, the TEM and SEM investigations are also necessary. Moreover, the dissolution method by organic solvents may also result in several new additions to our knowledge. In general it seems that during our experimental studies it is necessary to pay more attention to the pollen grains of the cultivated varieties.
2. The sphere as it is well known is an early form. From evolutionary point of view, the *Gramineae* type pollen grain may be originated morphologically from inaperturate *gymnospermous* ancestors, cf. *Spheripollenites* COUPER (1958). It was presumed previousy (KEDVES, TÓTH and FARKAS, 1991) that above the scheme of DOYLE (1977) concerning the evolutionary lineages of the *angiosperm* pollen grains, further, parallel lineage or lineages may also be presumed, within the *Normapolles* province.
3. The secondary forms, which appeared in consequence of the high temperature effect are very important from the point of view of the phylogeny of the *angiosperm* pollen grains. Here is the first place the appearance of the monosulcate form must be pointed out. The monosulcate pollen form is very common in several *gymnosperm* taxa. Trichotomosulcate pollen grains occur together with monosulcate ones often at the palms.

Finally, the monoporate – monosulcate alteration is important from the point of view of the fossil *Gramineae* pollen grains. In 1965 KEDVES described the *Monocolporopollenites* form-genus with two form-species from the Eocene layers in Hungary. KRUTZSCH (1970) established that the *Monocolporopollenites* fgen. may be synonymous to *Graminidites* COOKSON 1947 and published following new combinations:

G. grandiosus (KEDVES 1965) n. comb.
Monocolporopollenites grandiosus n. fsp. – KEDVES 1965, S. 336/7. Abb. 8, Taf. 3, Fig. 5 (Dorog-Gebiet/Ungarn; mittleres Eozän)
G. dorogensis (KEDVES 1965) n. comb.
Monocolporopollenites dorogensis n. fsp. – KEDVES 1965, S. 337, Abb. 10. Taf. 3, Figs. 6–7 (Dorog-Gebiet/Ungarn; mittleres Eozän)

Based on the results of our experiments, the taxonomical concept of KRUTZSCH (1970) has been verified. The *monocolporate* form may be a secondary alteration of the originally globular monoporate type.

Acknowledgements

This work was supported by the Grant OTKA 1/7, T 014692.

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